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THE INFLUENCE OF AN OXIDIZING SUBSTANCE (SODIUM IODOXYBENZOATE) ON IMMUNE REACTIONS *

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A review of the influence of chemical substances on immune reactions shows us that little has been done to determine the relation of oxidation to antibody formation, altho some of the results suggest a definite relationship between the two. Certain substances, which have the property of accelerating oxidation, have been found to have a stimulating effect on the production of antibodies, for example, the colloidal metals studied by Bossan and Marcelet[1] and Robin and Bordet,[2] which stimulate the production of opsonins. Strychnin has a stimulating effect on phagocytosis in vitro[3] as has also sodium iodoxybenzoate, an active oxidizing agent,[4] which stimulates the production of lysin in the dog, as shown by Hektoen.[5] Arsenic compounds also have a stimulating effect, as demonstrated by Agazzi[6] for agglutinin, Friedberger and Masuda[7] for hemolysin, and Strubell[8] for opsonin.

*Received for publication February 10, 1915.

1. Gaz. d. hôp., 1908, 81, p. 1227.
2. Comp. rend. Acad. d. sc., 1904, 138, p. 783.
3. Arkin: Jour. Infect. Dis., 1913, 13, p. 408.
4. Ibid., 1912, 11, p. 427.
5. Tr. Chicago Path. Soc., 1911, 8, p. 138.
6. Ztschr. f: Immunitätsf., 1909, 1, p. 736.
7. Therap. Monatsh., 1911, 25, p. 288.
8. Berl. klin. Wchnschr., 1912, 49, p. 1076.

We have evidence that substances which depress oxidations have an inhibitory effect on immune reactions. Numerous experiments have demonstrated that chloroform, ether, and alcohol have a detrimental effect on the natural defenses against infection (Rubin[9]) and interfere with the production of antibodies (Graham[10]). Morphin, potassium cyanid, and chloral have a depressing effect on phagocytosis (Arkin[3]), and chloral also prevents anaphylactic shock in the guinea-pig.[11] The fact that guinea-pigs protected by chloral do not become antianaphylactic indicates that this drug influences the mechanism of the allergic reaction. Some chemical substances which have no effect on the processes of oxidation in the body seem to have no marked effect on immune reactions. The antipyretics,[12] caffein,[3] and many other chemical substances have no influence on the production of antibodies.

In addition to this relationship between the effect of chemical substances on oxidation and their influence on the production of antibodies, we find a striking similarity between the activity of oxidizing ferments and the action of immune body and complement.[13] In both, three things are essential: A substance of a ferment nature; a substance on which it can act; and a substance which enables the ferment to act on the substrate and so to cause hydrolysis, oxidation, or some other type of reaction. Also, the ferment and the substrate are usually more specific to each other than to the third body which is simpler in nature, such as an alkali, an acid, a peroxid, or complement in immune serum. Finally, there is a similarity between the oxidizing ferments and immune sera in regard to thermostability. When an immune serum is heated to 55 C. the complement is destroyed but the immune body is unaffected. Likewise, when a vegetable extract is heated to 55 C. the peroxid is destroyed, but the ferment or peroxidase is untouched, and the inactivated juice can be reactivated by adding peroxid, just as the serum is reactivated by adding unheated serum containing complement.

Substances which are active oxidizing agents may influence the oxidation in the tissues by acting as catalysers. This action would not be due to the small amount of oxygen which they contain, but more likely to their catalytic effect, just as certain metals hasten oxidative processes. Most oxidizing agents cannot be used intravenously because they cause methemoglobin formation, gas embolism, or are not reduced in the body. For this reason, little is known regarding the reaction of cells to increased oxidation.

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[9] Jour. Infect. Dis., 1904, 1, p. 425; Jour. Amer. Med. Assn., 1907, 48, p. 1432.

[10] Jour Infect. Dis., 1911, 8, p. 147.

[11] Banzhaf and Famulener: Ibid., 1910, 7, p. 577.

[12] Kentzler and v. Benczur: Ztschr. f. klin. Med., Berl., 1909, 67, p. 242.

[13] Moore and Whitley: Biochem. Jour., 1909, 4, p. 136.

Sodium iodoxybenzoate, an organic peroxid which contains physiologically active oxygen and can be injected intravenously, offers an opportunity of studying the influence of oxidation on immune reactions. By increasing the intravital oxidations in the tissues it might have an effect on antibody production. The preparation of this compound has been discussed by me in a previous publication.[4] It is made from iodbenzoic acid, which is first oxidized to iodosobenzoic acid then to iodoxybenzoic acid. It contains two atoms of oxygen attached to the iodin in the molecule, thus possessing 11.43 percent of available oxygen. The oxygen can be determined by its power of liberating iodin from potassium iodid in acid solution.

The action of these substances was first studied by Heinz[14] in 1899. By the use of them, he attempted to study the effect of nascent iodin in the body. Our knowledge of the pharmacological action of sodium iodoxybenzoate we owe to Loevenhart[15] and his pupils who demonstrated that this compound oxidizes hemoglobin to oxyhemoglobin and can furnish oxygen for the peroxidase reaction by oxidizing phenolphthalin to phenolphthalein in the presence of blood. It causes a fall of blood pressure on intravenous injection by action on the vasomotor center, and it also acts on the respiratory center, producing apnea. These effects can be attributed to the oxygen attached to the iodin atom in the molecule, for iodbenzoic acid has no such action. Furthermore, the compound antagonizes the effect of hydrocyanic acid on the respiratory center. F. Jahn[16] has recently made a careful study of the effect of iodosobenzoic acid.

Sodium iodoxybenzoate has little effect on the blood. It causes a slight leukocytosis in about twenty-four hours after intravenous injection. This increase is in polymorphonuclears. The red blood cell count and hemoglobin are practically unchanged, as shown in the results, following the injection of 5 c.c. of a N/20 solution of sodium iodoxybenzoate intravenously into a rabbit.

Time	Hemoglobin Percent	Leukocytes	Erythrocytes
Before injection	90	15,000	6,100,000
6 hours after injection	85	18,000	5,900,000
24 hours after injection	85	20,000	5,700,000
90 hours after injection	90	16,000	6,000,000
120 hours after injection	90	16,000	6,000,000

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[14] Virchows Arch., 1899, 155, p. 44.

[15] Loevenhart and Grove: Jour. Phar. and Exper. Therap., 1911, 3, pp. 101, 131.

[16] Arch. f. exper. Path. u. Pharmakol., 1914, 76, p. 16.

These results agree with those of Loevenhart in his study of the pharmacologic action of sodium iodoxybenzoate.

That sodium iodoxybenzoate possesses oxygen in active form was demonstrated by me[17] in a study of its germicidal action toward various bacteria. These experiments showed that sodium iodbenzoate has very little bactericidal power as compared with that of the oxygen-containing substances. When the colon bacillus is used, the substances stand in the following relation to one another:

Substance	Oxygen Percent	Concentration Required to Kill	Relative Bactericidal Action
Sodium iodobenzoate	0.0	N/10 (2.7%)	1
Sodium iodosobenzoate	6.06	N/1000 (0.028%)	100
Sodium iodoxybenzoate	11.43	N/2000 (0.015%)	200

We see that the germicidal action of the oxygen-containing substances is dependent upon the physiologically active oxygen attached to the iodin. I found differences in the relative bactericidal value of sodium iodosobenzoate and iodoxybenzoate toward different organisms. These differences do not argue against the proposition that the germicidal action is due to the oxidizing action. A great many instances of specificity in oxidations with the simplest chemicals may be pointed out. If A and B each destroy C and D by oxidative processes, the fact that C is more sensitive to A than B does not indicate that D will also be more sensitive to A than B; indeed, the reverse is just as likely to be true. This statement holds whether C and D are living microorganisms or relatively simple chemicals.

We have further evidence in the action of similar organic peroxids in which the location of the oxygen atom or atoms in the benzene ring determines the activity of the compound as an oxidizing agent. The symmetrical organic peroxids with substitutions on both sides are inactive, the asymmetrical substitution products are active. There is here a promising field for chemotherapy, the discovery of organic peroxids with specific oxidizing properties or with specific activity toward certain bacteria. In this connection, I have found that the germicidal action of sodium iodoxybenzoate toward the four organisms which I studied varies inversely with the catalase value of the bacteria, i. e., the organism richest in catalase is least susceptible to the oxidizing agent. These results, which will be published later, would suggest that the enzyme catalase of bacteria protects them against active oxidizing agents and that the specificity may be due in part to the catalase content of the various bacteria studied.

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[17] Jour. Phar. and Exper. Therap., 1911, 3, p. 145.

Not only does sodium iodoxybenzoate have a marked germicidal action dependent upon its oxygen content, but I have found that it also has a marked stimulating action on phagocytosis in vitro.[4] This property is not possessed by sodium iodbenzoate which contains no oxygen. The opsonin of the serum is probably rendered more active by the oxygen-containing substance. Furthermore, the fact that potassium cyanid and many other substances, which exert an inhibitory effect on oxidations (alcohol, ether, chloroform, chloral, etc.), have a marked depressing effect on phagocytosis suggests a relationship between oxidation and the process of phagocytosis. If we agree with Verworn that ameboid movement is produced by decreased surface tension resulting from the introduction of oxygen into the living substance, we can readily see how the stimulating effect of sodium iodoxybenzoate on phagocytosis is produced.

That sodium iodoxybenzoate stimulates the production of antibody in immunized animals was first demonstrated by Hektoen.[5] He observed that dogs receiving the drug intravenously produced a greater amount of hemolysin than control animals or animals receiving sodium iodbenzoate. This demonstration led me to study the effect of sodium iodoxybenzoate on the production of various antibodies (hemolysin, agglutinin, and opsonin) in order to demonstrate, if possible, a relationship between the process of intravital oxidation and the mechanism of antibody formation.

ACTION OF SODIUM IODOXYBENZOATE ON THE PRODUCTION OF HEMOLYSIN

Rabbits immunized with ox corpuscles have been used in this work. Each rabbit was injected intravenously with 2.5 c.c. of washed ox corpuscles diluted with sufficient salt solution per kilogram of body weight. Fresh animals of about similar weights were selected (variation was not more than 75 gm.). Some of the rabbits then received an intravenous injection of 5 c.c. of neutral 1 percent sodium iodoxybenzoate one hour after the injection of the washed ox corpuscles. The sodium iodoxybenzoate used throughout this work was prepared by me and contained 11.2 percent of active oxygen when titrated with sodium thiosulphate. The control animals and those injected with the drug were then bled and the hemolytic value of the serum determined; 5 percent suspension of fresh ox corpuscles was used. The blood was obtained from the ear and the serum inactivated by heating to 56 C. for thirty minutes in a water bath. The dilutions were then made in sterile test tubes to which sufficient salt solution was added to make 1 c.c. in each. To each tube was added 0.05 c.c. guinea-pig complement, obtained by bleeding two guinea-pigs just before the experiment, and also 1 c.c. of a 5 percent suspension of fresh ox corpuscles. Controls were made with complement and corpuscles, amboceptor and corpuscles, and salt solution and corpuscles. The tubes were incubated at 37 C. for two hours and then placed in the ice-chest for twenty-four hours.

Chart 1 shows that sodium iodoxybenzoate has a stimulating effect on the production of specific hemolysin in rabbits injected with ox corpuscles, just as Hektoen found for dogs injected with goat corpuscles. The results indicate that the production of antibody is in some way connected with the process of oxidation, for a substance which is a physiologically active oxidizing agent stimulates antibody formation. Furthermore, sodium iodbenzoate, which contains no active oxygen, does not possess this property. This action of sodium iodoxybenzoate could not be due to the small amount of oxygen which is added to the blood, but more likely to the stimulating or catalytic effect which this organic peroxid exerts on the oxidations in the tissues, the site of antibody formation. Further evidence that this substance acts on the tissues rather than on the blood is presented in the discussion of the influence of this substance on the local allergic reaction.

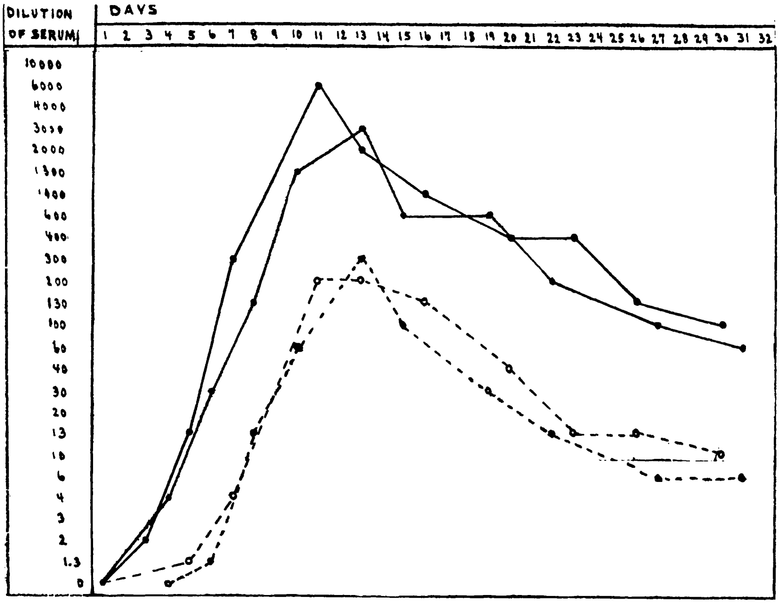

Chart 1.—The effects of sodium iodoxybenzoate on the production of specific hemolysin. Solid lines represent the specific lysin in blood of rabbits which on the first day received intravenous injection of 2.5 c.c. of washed ox corpuscles per kilogram of body weight, and one hour afterward 5 c.c. of 1 percent sodium iodoxybenzoate intravenously. The broken lines represent the specific lysin in blood of rabbits which on the first day received intravenous injection of 2.5 c.c. washed corpuscles per kilogram body weight.

Action of Sodium Iodoxybenzoate on the Production of Agglutinin

Rabbits of the same sex and species and practically the same weight and which had not been used for other work were injected with an old stock culture of the typhoid bacillus. Before the rabbits were used, the normal agglutinating power of the serum was determined.

Rabbits giving no agglutination of the typhoid bacillus in dilution of 1/20 before injection of typhoid bacilli were used for this work. Twenty-four-hour growths of a stock culture on standard agar slants were suspended in 0.85 percent sterile salt solution and killed by heating in a water bath at 60 C. for one hour. The rabbits received similar amounts of typhoid suspension, which in each case was equivalent to one twenty-four-hour slant agar growth, intraperitoneally. The control animal was given 5 c.c. of sterile salt solution intravenously, the other animal 5 c.c. of a sterile N/20 solution of sodium iodoxybenzoate intravenously, one hour after the injection of the typhoid bacilli. The animals were bled at intervals of two to four days and the agglutinating power of the serum determined. This was done by making dilutions of the serum as follows: 1/20, 1/40, 1/80, 1/160, 1/320, 1/640, 1/1,280, and 1/2,560. All the dilutions were made up to 1 c.c., and 1 c.c. of the bacterial suspension, made from living, twenty-four-hour cultures in 0.85 percent salt solution, was added. The mixtures were incubated two hours at 37 C. and then examined at the end of twenty-four hours. Only complete agglutination was recorded.

TABLE 1

The Influence of Sodium Iodoxybenzoate on the Production of Agglutinin

Animal	Agglutinating Power of Serum							
	3 Days	4 Days	6 Days	9 Days	11 Days	14 Days	17 Days	20 Days
Control	1/20	1/20	1/40	1/160	1/640	1/320	1/160	1/80
Iodoxybenzoate	1/40	1/40	1/160	1/320	1/2560	1/1280		

The experiment demonstrates that sodium iodoxybenzoate has a marked stimulating effect on the production of specific agglutinin in rabbits immunized with killed typhoid bacilli. On the eleventh day, the agglutinating power of the rabbit injected with sodium iodoxybenzoate was four times as great as that of the control animal.

The experiment was repeated with another pair of animals with practically similar results, altho in this second experiment I used only 4 c.c. of N/20 sodium iodoxybenzoate intravenously.

TABLE 2

The Influence of Sodium Iodoxybenzoate on the Production of Agglutinin

Animal	Agglutinating Power of Serum							
	3 Days	5 Days	7 Days	9 Days	11 Days	14 Days	17 Days	20 Days
Control	1/20	1/20	1/40	1/40	1/320	1/160	1/160	1/80
Iodoxybenzoate	1/20	1/40	1/80	1/160	1/1280	1/640	1/320	1/160

The fact that sodium iodoxybenzoate stimulates the production of agglutinin as well as hemolysin indicates that oxidation plays a rôle in the production of both and that there must be a close relationship between oxidation and antibody formation. Whether the tissue acted upon in the production of both of these antibodies is the same or not cannot be determined by these experiments. As I have already stated, this action of sodium iodoxybenzoate cannot be due to any effect on the blood, for the small amount of oxygen which it contains would have little influence on oxidative processes in the blood stream.

Experiments, the results of which will be published later, indicate that sodium iodoxybenzoate also stimulates the production of opsonin in rabbits injected with staphylococci.

THE INFLUENCE OF SODIUM IODOXYBENZOATE ON THE TUBERCULIN REACTION

The influence of sodium iodoxybenzoate on the local allergic reaction was studied by Amberg and Knox,[18] who found that this solution diminishes the intensity of the intracutaneous reaction when given intravenously in rabbits sensitized with horse serum. Iodbenzoic acid does not have this effect. These authors conclude that the solution does not influence the allergic reaction, but probably acts on the inflammatory processes because of a product of the allergic reaction. In a later study, Amberg[19] has observed that sodium iodoxybenzoate has an inhibitory effect on the inflammatory reactions produced by irritating substances, such as mustard oil and diphtheria toxin when given intracutaneously. These results indicate that the action of sodium iodoxybenzoate is on the early stage of the inflammatory reaction produced by toxic substances formed at the site of injection.

That the mechanism of general anaphylaxis is not the same as that causing the local allergic reaction on intracutaneous injection of an allergen in a sensitized animal is suggested by the work of Meigs,[20] who concludes that the intracutaneous reaction is produced by the action of substances formed at the site of injection, which cause the inflammatory reaction.

Assuming that the tuberculin reaction is similar in the mechanism of its production to the other local allergic reactions and is therefore produced by the formation of toxic substances at the site of injection, we should expect sodium iodoxybenzoate to have a similar effect on this inflammatory reaction as on those studied by Amberg. The following results indicate that this is the case, for sodium iodoxybenzoate has a marked inhibitory effect on the local tuberculin reaction in tuberculous animals.

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[18] Jour. Phar. and Exper. Therap., 1912, 3, p. 223.

[19] Ztschr. f. ges. exper. Med., 1913, 2, p. 19.

[20] Jour. Infect. Dis., 1914, 15, p. 541.

I have made a study of the influence of sodium iodoxybenzoate on the intracutaneous tuberculin reaction in some of the rabbits and guinea-pigs infected with well-developed, tuberculous lesions. The animals were placed at my disposal through the kindness of Dr. H. J. Corper. Those in each series of experiments had all been inoculated at the same time with similar amounts of bacilli. As controls were used tuberculous animals which were injected intracutaneously with 0.1 c.c. of human tuberculin, and this was immediately followed by the injection of salt solution, 0.85 percent. The control guinea-pigs were given the salt solution intraperitoneally; the rabbits intravenously. The treated animals were given similar quantities of the tuberculin and immediately afterward injected with neutral sodium iodoxybenzoate. The treated guinea-pigs received the drug intraperitoneally; the treated rabbits intravenously. The amounts of sodium iodoxybenzoate given to the treated animals and of salt solution given to the controls were 10 c.c. for the rabbits and 5 c.c. for the guinea-pigs. Measurements of the local reactions were made at the end of three, six, and twenty-four hours, by determining both the diameter and the thickness of the infiltration at the site of injection of the tuberculin. The measurements were made in millimeters with calipers.

The guinea-pigs were inoculated on November 27, 1912, with 0.02 mg. human tubercle bacilli in the inguinal region. On January 15, when intracutaneous tests were made, animal showed marked enlargement of local glands. Amount of tuberculin used was 0.1 c.c. of 1/5 human tuberculin intracutaneously in the shaved skin over abdomen. This was followed immediately by intraperitoneal injection of 5 c.c. of N/20 sodium iodoxybenzoate, and of 5 c.c. of 0.85 percent salt solution in control animal.

TABLE 3

EFFECT OF SODIUM IODOXYBENZOATE ON THE LOCAL TUBERCULIN REACTION IN GUINEA-PIGS INOCULATED IN THE INGUINAL REGION

Guinea-pig Injected with Salt Solution			Guinea-pig Injected with Sodium Iodoxybenzoate		
Hours	Diameter	Thickness	Hours	Diameter	Thickness
3	20 mm.	2.3 mm.	3	0 mm.	1.3 mm.
6	30 mm.	2.7 mm.	6	10 mm.	1.5 mm.
24	28 mm.	2.4 mm.	24	12 mm.	2.0 mm.
Thickness of normal skin 1.3 mm.			Thickness of normal skin 1.2 mm.		
Guinea-pig Injected with Salt Solution			Guinea-pig Injected with Sodium Iodoxybenzoate		
Hours	Diameter	Thickness	Hours	Diameter	Thickness
3	24.0 mm.	2.1 mm.	3	0 mm.	1.4 mm.
6	28.0 mm.	2.4 mm.	6	8 mm.	1.5 mm.
24	33.0 mm.	3.4 mm.	24	18 mm.	1.5 mm.
Thickness of normal skin 1.1 mm.			Thickness of normal skin 1.2 mm.		

Two more series of experiments were carried out with very similar results. In all there was a marked difference between the animal injected with sodium iodoxybenzoate and the control animal. The treated animals showed a marked diminution both in the diameter and thickness of the local area of inflammation. In two of the treated animals the reaction was so slight that it could be determined only by measuring the thickness of the skin fold at the site of injection.

The experiments were repeated in rabbits inoculated into the anterior chamber of the eye with human tubercle bacilli. In testing the influence of sodium iodoxybenzoate on the tuberculin reaction in these animals the drug was given by intravenous injections.

Rabbits were inoculated on November 15, 1912, with 0.02 mg. of human tubercle bacilli into the anterior chamber of the right eye. On January 24 when the intracutaneous tests were made, the animals showed distinct tubercle formation in the right eye. The length of time required for the tuberculin reaction to become positive following such an inoculation was determined. In an animal of this same series I found that the tuberculin test first became positive on January 7, seven weeks after the inoculation. One-tenth cubic centimeter of tuberculin was injected intracutaneously in the shaved skin over the abdomen. This was followed immediately by the injection of 10 c.c. of N/20 neutral sodium iodoxybenzoate intravenously in the treated animal and 10 c.c. of 0.85 percent salt solution in controls.

TABLE 4

Effect of Sodium Iodoxybenzoate on the Local Tuberculin Reaction in Rabbits Inoculated into the Anterior Chamber of the Eye

Rabbit Injected with Salt Solution			Rabbit Injected with Sodium Iodoxybenzoate		
Hours	Diameter	Thickness	Hours	Diameter	Thickness
3	10.0 mm.	1.6 mm.	3	0 mm.	1.4 mm.
6	18.0 mm.	2.0 mm.	6	10 mm.	1.6 mm.
24	30.0 mm.	3.5 mm.	24	15 mm.	2.0 mm.
Thickness of normal skin 1.3 mm.			Thickness of normal skin 1.4 mm.		

These results indicate that sodium iodoxybenzoate has a marked effect on the local tuberculin reaction in animals infected with tuberculosis. It diminishes the intensity of the reaction in guinea-pigs on intraperitoneal injection and also in rabbits on intravenous injection. If we assume that the local tuberculin reaction is a local inflammatory reaction produced as a result of the formation of toxic substances at the site of injection, which toxic substances are not produced in the normal animal, then the iodoxybenzoate may act by increasing the oxidative processes in the tissue in which the tuberculin is injected, so that the toxic substances are rendered less irritating because of a more rapid oxidation of the toxic substances. This would explain why the drug acts only in the early stages of the inflammatory reaction, also why repeated injections of iodoxybenzoate have no effect, as observed by Amberg for inflammatory reactions produced by toxin. For, after the toxic substances have combined with the tissues, the action of the drug would be prevented, and substances, such as cyanids, which prevent or reduce normal oxidation by the tissues, would tend to increase local inflammatory reactions by interfering with oxidation of toxic substances. Whether, in the case of the tuberculin reaction, the drug acts by accelerating oxidation of the tuberculin itself or of the toxic substances produced by the action of antibody or enzyme present in the sensitized animal on the tuberculin remains to be determined.

In order to determine whether or not sodium iodoxybenzoate acts on tuberculin in vitro by oxidizing it I made the following experiment:

Five cubic centimeters of 1 percent sodium iodoxybenzoate solution was placed in each of two test-tubes. To one, 1 c.c. of the tuberculin used was added, to the other 1 c.c. of 0.85 percent salt solution. In a third test tube 5 c.c. of salt solution and 1 c.c. of tuberculin were placed. The tubes were incubated at 37 C. for twenty-four hours. At the end of this time I determined the amount of oxygen present in each tube by the method which I use for determining the amount of available oxygen in the drug, i. e., by the liberation of I from KI in acid solution and titrating the iodin with N/10 sodium thiosulphate.

	N/10 Thio Required
Iodoxybenzoate 1 percent 5 c.c. + salt solution 1 c.c.	7.0 c.c.
Iodoxybenzoate 1 percent 5 c.c. + tuberculin 1 c.c.	5.2 c.c.
Salt solution 5 c.c. + tuberculin 1 c.c.	0.0 c.c.

These results show that tuberculin is oxidized by sodium iodoxybenzoate in vitro. In fact, 1 c.c. of the tuberculin combined with 25 percent of the available oxygen in 5 c.c. of 1 percent iodoxybenzoate. Calculating the amount of oxygen, I find that 1 c.c. of tuberculin combined with 1.44 mg. of oxygen (1 c.c. of N/10 thio corresponds to 0.0008 gm. of oxygen). Whether or not tuberculin treated with iodoxybenzoate in vitro produces a less marked local reaction or loses its power of producing the local inflammatory reaction in tuberculous animals as a result of its oxidation remains to be determined. Experiments now under way indicate that tuberculin is distinctly less toxic after its oxidation by certain agents. These results suggest the possibility of producing a tuberculin which has been deprived of its toxicity or whose toxicity has been reduced by the action of certain oxidizing agents. Such tuberculin might be of value in therapeutics if it does not lose its curative effect when its toxic action is reduced.

SUMMARY AND CONCLUSIONS

In previous publications I have demonstrated a relationship between oxidation and the process of phagocytosis, finding that substances which stimulate oxidations in vivo have a stimulating effect on phagocytosis, whereas substances which depress oxidations have an inhibitory effect on phagocytosis in vivo as well as in vitro. In order to study the influence of increased oxidation on other immune reactions, I have used sodium iodoxybenzoate, an organic peroxid, which has a marked effect on oxidative processes in the body. These properties are attributable to the oxygen combined with the iodin in the molecule. Sodium iodoxybenzoate has little effect on the blood and can therefore be injected intravenously. It has a stimulating effect on phagocytosis in vitro and possesses distinct germicidal properties.

Sodium iodoxybenzoate stimulates the production of hemolysin and agglutinin in rabbits when injected intravenously shortly after immunization. The results show that there is a close relationship between the production of antibodies and the oxidative processes. The substance probably acts by accelerating oxidations in the tissues which are the site of antibody formation. This acceleration is produced probably by some catalytic effect of the sodium iodoxybenzoate, for the small amount of oxygen injected into the circulation could not have this effect. That the action of this compound is exerted on the tissues is shown by the fact that the substance diminishes the intensity of the local allergic reaction in sensitized animals. It has a marked inhibitory action on the local tuberculin reaction in tuberculous animals. This action is due to a more active oxidation by the tissues of the toxic substances which cause the inflammatory reaction. Whether these toxic substances acted upon are the ones present in the tuberculin or are produced at the site of the inflammatory reaction in the tuberculous animal has not been determined. However, sodium iodoxybenzoate has the power of oxidizing tuberculin in vitro, and this oxidation is accompanied by a decrease in the toxicity when injected into normal animals.

All these results suggest that there is a close relationship between oxidation and immune processes, for a substance which is an organic peroxid stimulates the production of antibodies. It probably sets up in the tissues, which are the site of antibody formation, a more active oxidation, not the result of the amount of oxygen which it contains but because of some catalytic effect. That the compound can influence tissue oxidation is shown by its inhibitory action on the local allergic reaction, a reaction due, in part at least, to interference with oxidation at the site of the reaction.